MB16

```
5'- GATCC tctagagtcggc TTTACA ctttatgcttc(cg-gctcg..-3'
3'-     G agatctcagccg aaatgt gaaatacgaag gc(cgagc..-5'
   |    |             | -35|           |    |
    BamHI                               HpaII
```

MB22 insert

```
5'- GATCC actccccatccccctg TTGACA attaatcat  -3'
3'-     G tgaggggtaggggggac AACTGT taattagtagc-5'
   |    |                 | -35|           |
   BamHI                                   (HpaII)
```

Promoter and RBS variants of the fusion protein gene were constructed by basic DNA manipulation techniques to generate the following:

	Promoter	RBS	Encoded Protein.
pGEM-MB16	lac	old	VIIIs.p.-BPTI-matureVIII
pGEM-MB20	lac	new	''
pGEM-MB22	tac	old	''
pGEM-MB26	tac	new	''

The synthetic gene from variants pGEM-MB20 and pGEM-MB26 were recloned into the altered phage vector M13-MB1/2 to generate the phage constructs designated M13-MB27 and M13-MB28 respectively.

iii. Signal Peptide Sequence.

In vitro expression of the synthetic gene regulated by tac and the "new" RBS produced a novel protein of the expected size for the unprocessed protein (about 16 kd). In vivo expression also produced novel protein of full size; no processed protein could be seen on phage or in cell extracts by silver staining or by Western analysis with anti-BPTI antibody.